REMARKS

Applicants have amended the claims to make clear that the claimed process of the invention for isolating nucleic acids comprises the steps wherein nucleic acids in a sample are immobilized, released, and removed from one and the same side of a non-siliceous surface, and wherein the non-siliceous surface is present in a chamber of an isolation device such that the nucleic acid is both applied and removed from the same opening of the chamber of the isolation device. Accordingly, Applicants have amended Claim 1 to make clear that the claimed invention is a process for isolating nucleic acids of a sample that is applied to an isolation device. In particular, the process according to the invention for isolating nucleic acids comprises:

> providing an isolation device comprising:
>
> a generally cylindrical chamber defining a first opening at one end and a second opening at the other end, and
>
> a non-siliceous surface that is situated in said chamber between said first and second openings and having a first side facing said first opening and an opposing second side facing said second opening, and

that nucleic acids of a sample, immobilization buffer, wash buffer (if used), and elution agent are all applied to the first side of the non-siliceous surface through the first opening of the chamber, and further that nucleic acids are eluted from the non-siliceous surface and removed from the isolation device through the same (first) opening of the chamber. Such features of the claimed process using an isolation device are clearly described and exemplified in the specification (see, e.g., p. 6, lines 5-22, as examples of samples of nucleic acids useful in the claimed process; see, Figures 2-4; Brief Description of the Drawings at p. 13, lines 17-22; Detailed Description at p. 14, line 1-p. 16, line 8, describing isolation devices useful in claimed process, and as demonstrated using plastic column isolation devices in Examples 1-3 at p. 16, line 10-p. 22, line 4, and similarly employed in Examples 4-17 at p. 22, line 5-p. 39; of the specification). Accordingly, the amendments provide additional features of the claimed process as described in the specification and, therefore, add no new matter.

Applicants have also amended dependent Claims 2-5, 25, 32, and independent Claim 51 to clearly incorporate and recite relevant features and terms of the isolation device described in

Claim 1 in a consistent and coherent manner throughout the claims. Accordingly, the amendments to these claims add no new matter.

Applicants have amended Claim 9 to provide a preferred grammatical style to the claim language. The amendment adds no new matter.

Applicants have amended Claim 41 to recite the single broadest range of pore sizes for a membrane employed in the process according to any one of Claims 32-40. The other narrower ranges of pore sizes for membranes have been recited separately in new Claims 65 and 66. Thus, the amendment of Claim 41 along with new Claims 65 and 66 provide an alternative way to claim the subject matter in multiple claims. Accordingly, no new matter is added.

Entry of the amendments is respectfully requested.

Rejection Under 35 USC § 112, first paragraph

The Examiner rejected Claims 1-5, 9-22, 24-41, 44-50, and 59-64 under 35 USC § 112, first paragraph, as containing:

> "subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. No support for the exclusion of the species of ["siliceous"] surfaces from the genus of claimed surfaces has been found in the instant originally filed claims or specification. No citation or argument has been provided in the amendment of Paper [No.] 13 to support the amendment of claims 1, 4 and 5 reciting a surface which is "non-[siliceous]". Therefore, the term "non-[siliceous]" constitutes new matter." (section 11, pp. 3-4; of the Office Action, Paper No. 14).

For the reasons indicated below, Applicants respectfully traverse the rejection.

Respectfully, it appears that the Examiner's comments have provided an opinion but no legal basis for the rejection. Applicants have the right to claim any and all inventions disclosed in their specification. Siliceous and non-siliceous surfaces that may be employed in the invention are clearly described throughout the specification (see, e.g., p. 8, line 15-p. 9, line 20; Examples 1-19 at pp. 18-38; of the specification), and persons skilled in this art who read the specification would readily distinguish these two well-known categories of surfaces commonly employed in the nucleic acid biochemical protocols. Applicants have amended the claims in this

application to clearly and specifically cover the embodiment of the invention that employs an isolation device containing a non-siliceous surface.

Contrary to the Examiner's view, Applicants' specification provides a number of statements and examples that indicate or illustrate to persons skilled in this art that non-siliceous surfaces are particularly preferred over siliceous surfaces in the process of the invention. For example, the specification notes:

> "Less preferred, on the other hand, are fleeces such as silica fleeces, . . ." (p. 11, line 30; of the specification).

Example 1 (p. 16, line 10-p. 18, line 10; of the specification) compares yields of total RNA from lysed HeLa cells applied to plastic column isolation devices containing either hydrophilic nylon membranes or a silica membrane, the description of the results states:

> "The results of the two isolations with hydrophobic nylon membranes (Nos. 1 and 2) are shown in Table 1, compared with experiments in which on the one hand a hydrophilic nylon (Nyaflo) (No.3) *and a silica membrane* (No. 4) were used. The values reported in the table provide convincing support for the impressive isolation yield and separation effect of the materials used in accordance with the invention. They also show that silica gel-fleece produces clearly less yield, which can be attributed to its fleecelike structure and the ensuing absorption of a large portion of the eluted buffer." (p. 17, lines12-18; of the specification; emphasis)

> "Fig. 6 provides convincing evidence that when a silica membrane is used, no measurable proportion of the total RNA can be isolated." (p. 18, lines 9-10; of the specification)

Example 1 of the specification is then followed by Examples 2-16 that provide numerous studies and examples of the claimed process to isolate nucleic acids in a sample by employing isolation devices containing hydrophobic, non-siliceous surfaces, e.g., hydrophobic forms of nylon, polyesters, polyether sulfone, acryl copolymers, polytetrafluor ethylene, and polyvinylidene fluoride (see, e.g., Table 3, pp. 21-22; Table 4, p. 23; of the specification). Examples 17-19 provide other studies and examples of carrying out the claimed process to isolate nucleic acids in a sample employing isolation devices containing hydrophilic, non-

siliceous surfaces, e.g., hydrophilic forms of polyether sulfone, polyesters, polyamide, polyvinylidene fluoride, polycarbonate, and polypropylene fleece (see, e.g., Table 13, p. 37; Table 14, p. 39; of the specification). After many demonstrations and studies in these subsequent Examples of how to achieve excellent yields employing isolation devices containing non-siliceous surfaces, a final statement is provided in Example 19 that, again, expresses a preference for non-siliceous surfaces over siliceous surfaces in the claimed invention:

> "By using a silica membrane, no measurable amount of total RNA can be isolated if the eluate is taken from the membrane by drawing it off from the top." (p. 38, lines 17-18, of the specification)

Applicants submit that the above examples from the specification are more than sufficient to provide persons skilled in this art who read Applicants' specification with clear preference for practicing the invention using an isolation device containing a non-siliceous surface over a device containing a siliceous surface for isolating nucleic acids in a sample. Accordingly, the specification clearly supports Applicants' claims to the preferred embodiment of the invention using non-siliceous surfaces.

In view of the above comments and examples, Applicants respectfully submit that the rejection under 35 USC § 112, first paragraph, is improper and request that the Examiner reconsider and withdraw the rejection.

Rejection Under 35 USC § 102

In the Office Action, the Examiner rejected Claims 1-5, 9-15, 18-21, 24-26, 28-32, 36, 39-41, 50-52, 55, 58-60, and 62 as anticipated by US Patent No. 5,234,824 ("Mullis"). In particular, the Examiner stated:

> "Mullis teaches at the abstract, the summary and example 6, a process for isolating nucleic acids comprising charging a non-silicious surface (filter) with nucleic acid from the top of the surface. The nucleic acid is immobilized (trapped) on the surface of the filter, and released (eluted) off of the surface of the filter on the same side (top side) of the filter. The nucleic acid may be washed with a buffer solution. The buffer may contain metal ion (salt), a chaotropic agent (ammonium sulfate) or an alcohol. The filter is hydrophilic. The releasing solution may be water or a buffer solution which may contain a metal ion (salt), a chaotropic

> agent (ammonium sulfate) and an alcohol. The process may be done in a multiwell plate." (section 13, p. 4; of the Office Action)

For the reasons provided below, Applicants respectfully traverse the rejections.

For anticipation under 35 U.S.C. § 102 by a printed publication, that publication must teach each and every element or aspect of the claimed invention. As explained in § 2131 of the Manual of Patent Examining Procedure (MPEP):

> **"TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM**
>
> " 'A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). 'The identical invention must be shown in as complete detail as is contained in the . . . claim.' *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)." (emphasis in original).

As noted above, Applicants' invention provides processes for isolating nucleic acids in a sample comprising the use of *an isolation device* containing a non-siliceous surface *situated in a chamber* that defines a first opening at one end and a second opening at the other end of the chamber. The claimed invention also comprises the steps of charging one side of the non-siliceous surface in the chamber of the isolation device with nucleic acids of a sample applied through the first opening of the chamber, immobilizing the nucleic acids on the non-siliceous surface with an immobilization buffer applied through the same first opening, eluting the immobilized nucleic acids from the non-siliceous surface by applying an elution agent to the non-siliceous surface through the first opening of the chamber, and then removing the eluted nucleic acid through the same first opening of the chamber of the isolation device. Thus, in the claimed processes of Applicants' invention, *the non-siliceous surface on which nucleic acids are immobilized is static, remaining situated in the chamber of the isolation device*, while nucleic acids are immobilized on and eluted from the same side of the non-siliceous surface in the chamber and then removed from the isolation device *through one and the same (first) opening of the chamber of isolation device through which the nucleic acids were first applied to charge the non-siliceous surface.*

The above steps and properties of Applicants' invention are neither taught nor suggested by Mullis. Mullis teaches a method that requires gentle lysis of blood cells and avoidance of high shear forces to permit sufficiently high molecular weight DNA to be released and trapped in a membrane filter and further requires subsequent release of the DNA trapped on the filter by *physically transferring the filter into a cell culture dish, immersing and shaking the filter in an elution buffer added to the cell culture dish, and subsequently retrieving the DNA from the cell culture dish*:

> "The present invention contemplates releasing the DNA trapped on the filter by *immersing the membrane filter in a sufficient amount of distilled water* heated to about 100° C." (Summary of the Invention, col. 3, lines 36-39; of Mullis; emphasis added)
>
> "The filters *were removed* from the vacuum manifold *and placed into* 1 ml aliquots of an elution buffer containing 80 mM Tris-HCl at pH 9.0, 20 mM ammonium sulfate, 10 mM magnesium chloride *in the shallow well of CostarTM (Cambridge, Ma.) plastic cell culture dishes*. The dishes were placed on a rotary shaker for several hours at 37° C, at which time they were discovered to have evaporated to dryness. After addition of 1 ml aliquots of water, the dishes were placed back into the shaker for 30 minutes and aliquots taken for analysis." (Example 3, col. 9, lines 55-64; of Mullis; emphasis added)
>
> "Filter membranes retaining trapped DNA were prepared as in Example 3. *The filters were removed from the apparatus and each placed in a shallow well of a Costar TM plastic cell culture dish, and treated with one milliliter of elution buffer* as described in Table 3. Elution was performed at 42° C, with gentle rocking on a thermostated rotary shaker for 15 minutes at which point the liquid was decanted and analyzed at 260 nM and 280 nM for the appearance of DNA." (Example 6, col. 12, lines 28-36; of Mullis; emphasis added).

Hence, Mullis describes a method in which a vacuum manifold apparatus is dismantled to obtain a filter membrane that must be physically manipulated (immersion into a culture dish of buffer, rotary shaking) in order to obtain genomic DNA from the filter membrane. As noted above, Applicants' process does not involve dismantling an apparatus to obtain a non-siliceous surface to which nucleic acid is immobilized or transferring the non-siliceous surface to a culture dish of elution buffer that is shaken to elute the nucleic acid from the non-siliceous surface.

The differences in the procedure of Mullis and that of Applicant's invention is further evident by the fact that both sides of a membrane filter in Mullis necessarily contact DNA eluted from the membrane as the membrane is immersed and shaken in an elution buffer, *whereas in Applicants' process, the opposing (second) side of the non-siliceous surface situated in the chamber of the isolation device does not contact nucleic acids that are eluted and retrieved from the first side of the non-siliceous surface.* As explained in the specification, this feature permits buffer and other non-nucleic acid components that are applied through the first opening of the chamber to the first side of the non-siliceous surface to pass (e.g., by vacuum or gravity) to the opposing second side, i.e., the "waste side", of the non-siliceous surface and be expelled out through the second opening of the chamber (see, e.g., p. 11, lines 7-13; of the specification). This feature is particularly advantageous for carrying out Applicants' process in an automated format since a pipetting apparatus for applying sample and buffers and also for removing the desired eluate (isolated nucleic acids) need only be provided to the same first opening of the chamber and with respect to the one (first) side of the non-siliceous surface (see, e.g., p. 11, lines 13-21; of the specification). In addition, when Applicants' claimed process is carried out with the isolation device in a vertical position, the area above the non-siliceous surface may be used as a reaction area wherein the eluted nucleic acid may be subjected to one or more modification reactions, then re-immobilized for washing or separation from reaction reagents, and finally re-eluted for removal of the modified nucleic acids through the one and same (first) opening of the chamber (see, e.g., p. 12, lines 3-12; of the specification; Claim 9). Such advantages and steps of Applicants' claimed process are neither taught nor suggested, nor even attainable, in the process of Mullis.

The above comments illustrate the clear differences in the method of Mullis for isolating high molecular weight DNA and Applicants' claimed process for isolating nucleic acids in a sample. Applicants submit that Mullis clearly fails to teach each and every element of Applicants' claimed invention, and, thus, fails as a reference to reject Applicants' claims as lacking novelty under 35 USC § 102. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Rejections Under 35 USC § 103

In the Office Action, the Examiner rejected Claims 1-5, 9-15, 18-22, 24-32, 36, 39-41, 44-52, 55, and 58-63 as obvious over Mullis in view of US Patent No. 6,028,186 ("Tasset"). The Examiner applied the primary reference Mullis as teaching Applicants' as in the rejection under 35 USC § 102, but further states:

> "Mullis did not teach that the process buffer may contain phenol or various chaotropic agents in claims 27 or 45 which may be in the immobilization buffer.
>
> "Tasset et al. teach at columns 19-20 the isolation of nucleic acids on a surface and the release of the nucleic acids from the surface where the immobilization buffer, wash buffer or elution buffer may contain urea (one of the instant claimed chaotropic agents) and phenol.
>
> "It would have been prima facie obvious to one of ordinary skill in the art at the time of filing the instant application to combine the teachings of Mullis with Tasset et al. because each of Mullis and Tasset et al. teach a process for isolating nucleic acids comprising charging a non-silicious surface with nucleic acid from the top of the surface *where the nucleic acid is immobilized on the surface of the filter, and released off of the same side of the surface.* The teachings of Tasset et al. make obvious the modification of the method by using an immobilization buffer, wash buffer or elution buffer which may contain urea (one of the instant claimed chaotropic agents) and phenol." (section 15, pp. 6-6; of the Office Action)

Applicants respectfully traverse the rejection for the reasons provided below.

As discussed above, the primary reference Mullis describes a process for obtaining genomic DNA by applying a lysate of blood cells to a filter membrane in a vacuum manifold apparatus, physically dismantling the apparatus to obtain the filter membrane to which genomic DNA has been applied, transferring the filter membrane to a cell culture dish, immersing the filter in an elution buffer in the culture dish, and incubating the culture dish on a rotatory shaker to elute the genomic DNA from the filter membrane (see, e.g., col. 9, lines 55-64; col. 10, lines 44-45; col. 12, lines 33-36; of Mullis). In contrast, Applicants' claimed process for isolating nucleic acids in a sample employs an isolation device comprising a non-siliceous surface that is

situated in a chamber defining two opposing openings so that the non-siliceous surface is *never* transferred from the chamber of the isolation device, and nucleic acids in a sample are applied to and removed from one and the same side of the non-siliceous surface through one and the same ("first") opening of the chamber. Clearly, Mullis fails to teach or suggest Applicants' claimed process.

Tasset does not cure the deficiencies of Mullis to make Applicants' claimed process obvious. Nowhere does Tasset describe Applicants' claimed process. The inventive feature of Applicants' claimed process does not reside solely in using an immobilization buffer comprising a strong chaotropic agent, such as urea (e.g., Claims 27, 45), or comprising a phenol (e.g., Claim 22; Example 13, p. 31, lines 5-15; of the specification). Notwithstanding this point, persons skilled in this art would recognize that, unlike Applicants' process, the process described in Tasset ***cannot*** employ a buffer containing urea and/or phenol to immobilize nucleic acid molecules to a filter membrane. The reason for this prohibition is that Tasset describes use of the SELEX procedure (US Patent No. 5,270,163, "Gold") for synthesizing and selecting high affinity RNA ligands that bind a target protein (e.g., IFN-gamma in Tasset). The SELEX procedure in Tasset relies on a partitioning phenomenon that is well known in nucleic acid molecular biology, i.e., under proper conditions protein/nucleic acid ligand *complexes* (e.g., protein/RNA ligand complexes in the SELEX procedure) will bind nitrocellulose filters whereas free nucleic acid (e.g., RNA not complexed with a protein ligand in the SELEX procedure) will not be effectively retained on such filters and, therefore, can be readily removed from or washed through such filters (see, e.g., col. 24, line 65-col. 25, line 3; col. 30, lines 52-53; col. 34, lines 19-30; col. 35, lines 26-31; col. 37, lines 38-43; of Gold in Exhibit A). Thus, RNA in protein/RNA ligand complexes bound to nitrocellulose filters can be *released* from such filters by immersing or bathing the filters in a buffer that will disrupt the protein/RNA ligand complex, e.g., using a chaotrope such as urea (e.g., 7 M) and/or a protein denaturing agent such as phenol (see, e.g., col. 25, line 67-col. 26, line 20; of Gold in Exhibit A; col. 20, lines 35-46 of Tasset). Thus, an "immobilization buffer" containing urea and phenol as envisioned by the Examiner is ***not at all*** taught in Tasset or Gold as such buffers would ***prevent*** immobilization of relevant protein/RNA ligand complexes, and thereby, prevent isolation of the RNA sought by the method in Tasset. Applicants' process for isolating nucleic acids in a sample does not rely on the partitioning phenomenon described in Tasset and, to the contrary, is able to provide excellent yields using

immobilization and wash buffers comprising the very denaturing compounds (see, e.g., Examples 1-19; of the specification) that would render the process described in Tasset inoperable. Clearly, Tasset does not describe the immobilization and wash buffers used in Applicants' invention or the steps employing the isolation device containing a non-siliceous surface as required in Applicants' claimed process and lacking in Mullis. Accordingly, Tasset does not cure the deficiencies of Mullis to provide persons of ordinary skill in this art with Applicants' claimed process.

In view of the above explanation and Exhibit A, Applicants respectfully submit that the combination of Mullis with Tasset clearly does not provide a case of *prima facie* obviousness to reject Applicants' claims as required by 35 USC § 103. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

The Examiner also rejected Claims 1-5, 9-15, 18-22, 24-36, 39-41, 44-52, 55, and 58-63 as obvious over Mullis in combination with Tasset and WO 97/08547 ("Su"). The Examiner relied on Mullis and Tasset as in the earlier rejection and further applied Su because:

> "Mullis and Tasset et al. did not teach that the membrane may be *hydrophobic* (various types of *hydrophobic* membranes).
>
> "Su teach at the summary at pages 3-7, example 1 and claims 29-37 the use of *hydrophobic* membranes, and hydrophobized membranes in a method of binding and releasing nucleic acids from the surface of a membranes [sic] with immobilization buffers, washing buffers and releasing buffers containing ionic metal salts, organic acid salts, or hydroxyl derivatives of aliphatic hydrocarbons (alcohols). . . .
>
> "One of ordinary skill in the art would have been motivated to combine the teachings of Mullis, Tasset et al. and Su because Su at page 2, [sic] the desirable and beneficial use of the *hydrophobic* surfaces to improve the immobilization (section 16, pp. 7-8; of the Office Action; emphasis added)

Applicants respectfully traverse the rejection based on the comments below.

At the outset, Applicants note that Su describes the use of a matrix comprising a ***hydrophilic***, not hydrophobic, organic polymer in a method and apparatus to isolate nucleic acids (see, Abstract, Summary at pp. 3-7, Example 1 (cellulose, e.g., from Whatman 3 MM paper), Claims 1-37; of Su). Thus, Su does not provide the literal support for the Examiner's rejection.

Applicants refer to their comments above for noting the deficiencies in the combination of Mullis and Tasset as a basis for rejecting Applicants' claims as *prima facie* obvious. With respect to Su, even assuming, *arguendo*, that the Examiner meant to rely on Su as a reference for using a hydrophilic matrix to isolate nucleic acids, Su still fails at a more fundamental level to cure the deficiencies of Mullis and Tasset. The inventive feature of Applicants' invention does not rest in the fact that hydrophilic or hydrophobic surfaces were known or employed in various laboratory procedures prior to the invention; Applicants have stated that examples of such materials are known and have been readily available from a variety of commercial sources for many years (see, Examples 1-19; of Applicants' specification). In Su, the hydrophilic solid matrix (processed from Whatman 3 MM filter paper) is employed in standard forms, e.g., in a column (see, p. 9, lines 2-14; Examples 1-4; of Su), batch matrix (see, Example 5, of Su), or coated on beads (see, Example 9, of Su). Using the matrix in batch or as matrix-coated beads in Su requires physical manipulation of the matrix material, which, as mentioned with respect to Mullis above, is clearly not involved in Applicants' claimed process as explained above. With respect to matrix-packed columns, nowhere does Su provide a description or appreciation for applying and recovering nucleic acids from the same side of the matrix surface and from the same opening of the column device. On the contrary, Su only refers to recovering nucleic acid that is eluted and that "flows through" the matrix and column (see, p. 42, lines 9-19 and Fig.1; of Su). Accordingly, Su does not describe, suggest, or contemplate Applicants' claimed process employing an isolation device in which nucleic acid is applied to and recovered from the same side of a non-siliceous surface in the device and from the same opening of the chamber in which the non-siliceous surface is situated.

In view of the above comments, Applicants submit that it is clear that Su does not cure the deficiencies of the combination of Mullis and Tasset to provide a case of *prima facie* obviousness to reject Applicants' claimed invention under 35 USC § 103. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection.

The Examiner also relied on the combination of Mullis, Tasset, Su, and also EP Publication No. 0 597 951 A1 ("Raybuck") and US Patent No. 5,869,073 ("Sawan") to reject Claims 1-5, 9-15, 18-22, 24-41, 44-55, and 58-64 as obvious under 35 USC § 103. For the reasons provided below, Applicants respectfully traverse the rejections.

The Examiner relies on Mullis, Tasset, and Su as in the previous rejections. The Examiner further relies on Raybuck because:

> "Raybuck et al. teach at page 5 and the claims, the use of hydrophobic nylon membranes, and hydrophobized membranes in a method of binding nucleic acids on the surface of the membranes with immobilization buffers and washing buffers." (section 17, p. 9; of the Office Action)

The Examiner also relies on Sawan because:

> "Sawan et al. teach at column 7, lines 18-39 and column 9, lines 32-59 the use of hydrophilic and hydrophobic membranes and hydrophilized and hydrophobized membranes, which may be nylon, in the capture and release of nucleic acids from the surface of the membrane." (section 17, p. 9; of the Office Action)

Applicants refer to the previous comments for explaining the various deficiencies of Mullis, Tasset, and Su with respect to Applicants' claimed process. In addition, to the extent the Examiner has cited Raybuck and Sawan as teachings of hydrophilic and hydrophobic membranes *per se*, Applicants again note that the inventive feature of their claimed process does not reside in whether a hydrophilic, hydrophobic, hydrophilicized, and/or hydrophobicized membrane was previously known in the art; they were. As in the case of Su above, Applicants note that neither Raybuck nor Sawan provide any description of Applicants' process comprising use of an isolation device wherein nucleic acids in a sample are applied to and recovered from a single side of a non-siliceous surface situated in a chamber of the isolation device and wherein the nucleic acids are applied to and recovered from the non-siliceous surface through one and the same opening of the chamber of the isolation device.

Raybuck describes a method of isolating intact nuclei (or other organelles) that relies on the formation of a mat or mesh of DNA released from a small portion of lysed nucleic on a membrane that is able to gently trap and support a large portion of unlysed nuclei from eukaryotic cells (see, e.g., p. 5, lines 5-6; Claim 1; of Raybuck). The nuclei may be released by degrading the DNA mesh, e.g., by nuclease digestion, followed by physically manipulating the membrane containing the nuclei and DNA mesh, e.g., by washing or centrifuging the nuclei

away from the DNA mesh (see, e.g., p. 5, lines 14-25; of Raybuck). Clearly, Raybuck describes a procedure and a goal (isolation of intact nuclei) totally distinct from Applicants' process.

Sawan describes anti-microbial filters produced by coating a filter with a composition comprising a non-metallic, anti-microbial compound, such as a biguanide polymer, and an anti-microbial metal (see, e.g., col. 8, line 6-col. 9, line 7; col. 10, line 65-col. 11, line 26; of Sawan). The filters may be any of a variety of known hydrophobic or hydrophilic membranes (see, col. 7, lines 18-39; of Sawan) or membranes having a combination of hydrophobic and hydrophilic regions (see, col. 9, lines 32-59; of Sawan). The anti-microbial filters of Sawan are particularly useful as components of multi-dose liquid dispensing containers designed to prevent external microbial contamination of the liquid during repeated use (see, e.g., col. 1, lines 63-65; Figures 1-5; of Sawan). Clearly, Sawan does not describe any method for isolating nucleic acids of interest from a sample, and certainly does teach or suggest Applicants' claimed process.

Raybuck and Sawan fail to advance the combination of Mullis, Tasset, and Su to provide the art with Applicants' claimed process of isolating nucleic acids in a sample. Raybuck and Sawan do not even apprehend any need for or advantage of Applicants' process. Raybuck and Sawan only provide documents that mention the existence of hydrophilic and hydrophobic membranes that have been employed over the years in various laboratory and manufacturing procedures. Not only does the combination of documents relied on by the Examiner provide an inadequate cumulative list of the critical features of Applicants' invention, but attempts at piecemeal hindsight reconstruction of an invention is repugnant to the patent law and expressly prohibited. *See, In re Kotzab*, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316-17 (Fed. Cir. 2000).

In view of the above comments, Applicants respecfully submit that the combination of Mullis, Tasset, Su, Raybuck, and Sawan clearly fails to provide a basis to reject Applicants' claims as *prima facie* obvious as required by 35 USC § 103. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejection.

The Examiner also rejected Claims 1-5, 9-15, 18-22, 24-41, 44-55, and 58-64 as obvious over the combination of Mullis, Tasset, Su, Raybuck, and Sawan, and further in view of US Patent No. 5,728,531 ("Yamada"). The Examiner applies Mullis, Tasset, Su, Raybuck, and Sawan as in the previous rejections noting:

> "Yamada et al. teach at example 4, the use of membranes, in a method of binding nucleic acid on the surface of the membranes with immobilization buffers and washing buffers.
>
> "It would have been prima facie obvious to one of ordinary skill in the art at the time of filing the instant application to combine the teachings of Mullis, Tasset et al., Su, Raybuck et al., Sawan et al. and Yamada et al. because each of Mullis, Tasset et al., Su, Raybuck et al., Sawan et al. and Yamade et al. teach a process for isolating nucleic acids comprising charging a non-silicious surface with nucleic acid from the top of the surface where the nucleic acid is immobilized on the surface of the filter, using buffers for immobilization and washing. *The teachings of Yamada et al. make obvious the modification of the method by using buffers which contain citric acid in the capture of nucleic acids on the surface of the membrane.*
>
> "One of ordinary skill in the art would have been motivated to combine the teachings of Mullis, Tasset et al., Su, Raybuck et al., Sawan et al. and Yamada et al. because the citric acid buffer taught by Yamada et al. is desirable and beneficial to use in a method of binding nucleic acids to membranes and is a well known and obvious choice which is available to one of ordinary skill in the art for practicing the method. Further, a person of ordinary skill in the art would have had a reasonable expectation of success in the producing [of] the instant claimed invention given the teachings of Mullis, Tasset et al., Su, Raybuck et al., Sawan et al., and Yamada et al." (section 18, pp. 11-12; of the Office Action; emphasis added).

For the reasons provided below, Applicants respectfully traverse the rejection.

Applicants refer to their above comments for delineating the deficiencies of Mullis, Tasset, Su, Raybuck, and Sawan, alone and in combination. As noted in the above excerpt from the Office Action, the Examiner relies on Yamada for providing to the Examiner's combination of documents, a description of using citric acid buffers in immobilizing nucleic acids to membranes. Applicants' claimed process may indeed be carried out using an immobilization buffer based on a polyhydroxycarboxylic acid, such as citric acid buffer (see, e.g., Example 12, Table 10, p. 30, line 5-p. 31, line 4; of the specification), however, the inventive features of Applicants' claimed process have been explained above and clearly do not rest on the use of a citric acid-based immobilization buffer. Yamada describes gibberellin-labeled DNA probes for *detecting* a target nucleic acid immobilized on a solid support by a standard hybridization

reaction. The hybridization reaction between a gibberellin-labeled DNA and a nucleic acid immobilized on a solid support may be carried out using a citric acid buffer (see, e.g., Example 14, col. 10, lines 37-41; of Yamada). The gibberellin-labeled DNA probes of Yamada that hybridize to a target nucleic acid immobilized on the solid support can then be detected using anti-gibberellin antibody labeled by any of a number of molecules commonly employed for immunodection systems (see, e.g., col. 3, line 58-col. 4, line 17; of Yamada). Clearly, Yamada does not describe a process for isolating nucleic acids according to Applicants' invention.

The above comments show that combining Yamada with the other documents cited by the Examiner still cannot provide persons of ordinary skill in this art with a description of Applicants' claimed process for isolating nucleic acids in a sample. Accordingly, the combination of Mullis, Tasset, Su, Raybuck, Sawan, *and* Yamada fails to render Applicants' claims obvious under 35 USC § 103. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Conclusion

The above comments and Exhibit A clearly show that Applicants' claimed invention is fully supported by the specification and that none of the documents cited by the Examiner, alone or in any combination thereof, teaches or suggests Applicants' claimed process for isolating nucleic acids in sample. Accordingly, Applicants submit that the claims, as amended herein, are now in condition for allowance and respectfully request that the Examiner enter the amendments, withdraw the rejections, and pass the claims to allowance for grant of Letters Patent.

Respectfully submitted,

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